

CLAIMS

1. A method of increasing yield of a protein from a cell culture, comprising:
 - (a) causing a pseudo-senescent state in one or more cells in the cell culture; and
 - (b) collecting a protein fraction from the cell culture.
- 5 2. The method of claim 1, wherein the protein is a secreted protein.
3. The method of claim 2, wherein the protein is an antibody.
4. The method of claim 2, wherein the protein fraction is collected from the cell culture medium.
- 10 5. The method of claim 1, wherein the protein is an intracellular protein.
6. The method of claim 1, wherein the protein is a membrane protein.
7. The method of claim 1, wherein the cell culture is a eukaryotic cell culture
8. The method of claim 1, wherein the cell culture is a mammalian cell culture.
- 15 9. The method of claim 8, wherein the mammalian cell is contact-dependent for growth.
10. The method of claim 9, wherein the mammalian cell is a hybridoma.
11. The method of claim 1, wherein the pseudo-senescent state is caused by contacting a cell with a composition that inhibits cell proliferation.
- 20 12. The method of claim 11, wherein the composition comprises an expression vector.
13. The method of claim 11, wherein the expression vector comprises an inducible transcription regulation element.

14. The method of claim 13, wherein the transcription regulation element comprises a tetracycline operator element.
15. The method of claim 14, wherein the transcription regulation element comprises a plurality of tetracycline operator elements.
- 5 16. The method of claim 15, wherein the tetracycline operator elements are arranged such that two phased tetracycline operators are downstream from a TATA sequence and two phased tetracycline operators are upstream of the TATA sequence.
- 10 17. The method of claim 12, wherein the expression vector encodes a cyclin-dependent kinase inhibitor.
18. The method of claim 17, wherein the cyclin-dependent kinase inhibitor is a Cip/Kip family member.
19. The method of claim 18, wherein the Cip/Kip family member is selected from the group consisting of p21, p27, and p57.
- 15 20. The method of claim 17, wherein the cyclin-dependent kinase inhibitor is a INK4 family member.
21. The method of claim 20, wherein the INK4 family member is selected from the group consisting of p15, p16, p18, and p19.
22. The method of claim 1, wherein the pseudo-senescent state is caused by expression of one or more proteins encoded by one or more expression vectors.
- 20 23. The method of claim 22, wherein the proteins are p16, p21, and p57.
24. The method of claim 23, wherein the proteins are p16, p21, and p27.

25. A method of increasing yield of a protein from a eukaryotic cell culture, comprising:

- (a) contacting the cell culture with an expression vector which comprises an inducible transcription regulation element comprising a tetracycline operator element; and
- (b) collecting a protein fraction from the cell culture.

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26. A transcriptional regulatory element comprising:

- (a) a minimal promoter comprising a TATA sequence;
- (b) two phased tetracycline operators downstream from the TATA sequence; and
- (c) two phased tetracycline operators upstream of the TATA sequence.

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27. The transcriptional regulatory element of claim 26, wherein the two phased tetracycline operators downstream from the TATA sequence are 21 basepairs downstream from the TATA sequence.

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28. The transcriptional regulatory element of claim 26, wherein the two phased tetracycline operators upstream from the TATA sequence are 11 basepairs upstream from the TATA sequence.

29. The transcriptional regulatory element of claim 26, wherein:

- (a) the two phased tetracycline operators downstream from the TATA sequence are 21 basepairs downstream from the TATA sequence; and
- (b) the two phased tetracycline operators upstream from the TATA sequence are 11 basepairs upstream from the TATA sequence.

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30. The transcriptional regulatory element of claim 26, wherein the minimal promoter is a CMV promoter.

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31. An expression vector comprising:

- (a) a minimal promoter comprising a TATA sequence;

- (b) two phased tetracycline operators downstream from the TATA sequence; and
- (c) two phased tetracycline operators upstream of the TATA sequence.

32. The expression vector of claim 31, wherein the two phased tetracycline operators downstream from the TATA sequence are 21 basepairs downstream from the TATA sequence.

33. The expression vector of claim 31, wherein the two phased tetracycline operators upstream from the TATA sequence are 11 basepairs upstream from the TATA sequence.

34. The expression vector of claim 31, wherein:

- (a) the two phased tetracycline operators downstream from the TATA sequence are 21 basepairs downstream from the TATA sequence; and
- (b) the two phased tetracycline operators upstream from the TATA sequence are 11 basepairs upstream from the TATA sequence.

35. The expression vector of claim 31, wherein the minimal promoter is a CMV promoter.

36. The expression vector of claim 31, wherein the vector is a viral vector.

37. The expression vector of claim 36, wherein the viral vector is a retroviral vector.

38. The expression vector of claim 37, wherein the retroviral vector is a Moloney strain murine leukemia virus vector.

39. The expression vector of claim 31, further comprising a gene operably linked to the promoter.

40. The expression vector of claim 39, wherein the gene encodes a cyclin dependent kinase inhibitor.

41. The expression vector of claim 40, wherein the cyclin dependent kinase inhibitor is selected from the group consisting of p21, p27, p57, p15, p16, p18, and p19.
42. The expression vector of claim 41, wherein the vector encodes more than one cyclin-dependent kinase selected from the group consisting of p21, p27, p57, p15, p16, p18, and p19.
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